

IN THE CLAIMS:

Please cancel pending claims 1, 5-22, 25, 35, 39-41, 44, 45, 48 and 49, without prejudice.

Please add new claims 50-77, as follows:

50. A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, comprising:

a) a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron;

b) a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter;

c) optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter; and,

d) a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter and wherein at least either the first, second or third cistron encodes at least one immunogenic epitope of a human immunodeficiency virus antigen.

51. The polynucleotide of Claim 50 wherein the first cistron encodes a human immunodeficiency virus (HIV) gene selected from the group consisting of env, gag, gag/pol, gag/protease, gag and portions of pol not encoding a functional polymerase, and pol.

52. The polynucleotide of Claim 50 wherein the second cistron encodes a human immunodeficiency virus (HIV) REV gene if the first cistron encodes an HIV gene, the efficient expression of which is dependent on availability within the cell expressing the HIV gene of the REV gene product.

53. The polynucleotide of Claim 52 wherein the first cistron encodes an HIV late gene selected from env, gag and pol.

54. The polynucleotide of Claim 53 wherein the first cistron encodes HIV gp160, HIV gp120, HIV gp41, HIV gp120 lacking a CD4 binding site and HIV env with an immunologically altered V3, the altered V3 having an altered glycosylation pattern or substituted V3 loop tips.

55. The polynucleotide of Claim 52 wherein the third cistron encodes a cytokine or a T-cell costimulatory element.

56. The polynucleotide of Claim 55 wherein the cytokine is interferon, GM-CSF, or interleukin.

57. The polynucleotide of Claim 55 wherein the T-cell costimulatory element is a gene encoding a B7 protein.

58. The polynucleotide of Claim 50 wherein the first cistron encodes a REV-independent human immunodeficiency (HIV) epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination.

59. The polynucleotide of Claim 58 wherein the second cistron encodes an interleukin, an interferon, or GM-CSF, and the third cistron encodes a B7 protein.

60. The polynucleotide of Claim 50 wherein either of the second and third cistron is under transcriptional control of the transcriptional promoter upstream of the first cistron, a sequence is provided upstream of each of the second and third cistrons having the function of an internal ribosome entry site (IRES) to effect efficient translation of the second and third cistrons on a bi- or tri-cistronic messenger RNA transcribed from the beginning of the first cistron through each of the second and third cistrons up to the transcriptional terminator following the second or third cistron.

61. The polynucleotide of Claim 60 wherein the IRES is selected from encephalomyocarditis virus (EMCV) IRES, swine vesicular virus IRES and poliovirus IRES.

62. The polynucleotide of Claim 60 wherein the first cistron encodes a human immunodeficiency virus (HIV) REV dependent gene, the second cistron encodes REV, and the third cistron encodes a T-cell costimulatory element or a cytokine, and further, wherein the first cistron is preceded by a transcriptional promoter and the second and third cistrons are each preceded by an IRES and no transcriptional promoter.

63. The polynucleotide of Claim 62 wherein the first cistron encodes an HIV gp160, the first cistron is preceded by cytomegalovirus immediate early promoter, the second cistron encodes HIV REV, the optional third cistron encodes an interferon, GM-CSF, an interleukin, or a B7 protein.

64. A polynucleotide which cannot replicate in eukaryotic cells *in vivo* and which comprises contiguous nucleic acid sequences capable of being expressed to produce a gene product upon introduction of the polynucleotide into eukaryotic tissues *in vivo*, wherein the gene product either acts as an immunostimulant or as an antigen capable of generating an immune response, wherein the nucleic acid sequences encode:

- a) a spliced REV gene;
- b) a spliced human immunodeficiency virus (HIV) immunogenic epitope; and,
- c) optionally, a cytokine or a T-cell recognition element.

65. The polynucleotide of Claim 64 wherein the HIV immunogenic epitope of step b) is a gene product expressed from an HIV gene selected from the group of HIV genes consisting of gag, gag-protease, and env or an immunogenic subportion thereof; the cytokine is interleukin-12, and the T-cell costimulatory element is a B7 protein

66. The polynucleotide of Claim 65 wherein the env immunogenic epitope is a gene product expressed from an env open reading frame selected from the group consisting of HIV gp160, HIV gp120 and HIV gp41.

67. The polynucleotide of Claim 65 wherein the gag immunogenic epitope is p17, p24, or p15.

68. A polynucleotide comprising a first gene encoding an HIV gag, gag-protease, or env immunogenic epitope, the first gene containing a REV responsive element (RRE) or having been modified to contain an RRE, the first gene being operatively linked with a transcriptional promoter suitable for gene expression in a mammal, the first gene being linked with an internal ribosome entry site (IRES), and the IRES being linked with a second gene encoding a REV gene product, wherein said polynucleotide is non-replicating in eukaryotic cells *in vivo*.

69. A method for co-expression in a single cell *in vivo*, of at least two gene products, which comprises introducing between about 1 ng and about 100 mg of the polynucleotide of Claim 50 into the tissue of a mammal.

70. A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising:

- a) a eukaryotic transcriptional promoter;
- b) an open reading frame 3' to the transcriptional promoter encoding an immunogenic HIV epitope wherein the open reading frame has a splice donor sequence at the 5'-side of the open reading frame, a REV responsive element anywhere within the open reading frame, and a stop codon encoding the termination of translation of the open reading frame;
- c) an internal ribosome entry site (IRES) 3' to the translation stop codon of the open reading frame;
- d) an open reading frame encoding a spliced HIV REV gene at the 3' end of which is a translation stop codon;
- e) optionally, 3' to the REV translation stop codon, a second IRES, followed by an open reading frame encoding immunomodulatory or immunostimulatory genes being selected from the group consisting of GM-CSF, IL-12, interferon, and a B7 protein; and,
- f) a transcription-termination signal 3' of the most downstream open reading frame of step d) or optionally, step e).

71. A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

- a) a eukaryotic transcription initiation signal;
- b) an HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;
- c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the HIV ORF;
- d) a sequence encoding an ORF of a T-cell costimulatory element 3' to the IRES; and
- e) a transcription termination signal 3' to the translation stop codon of the T-cell costimulatory element.

72. The polynucleotide of Claim 71 wherein the HIV gene ORF in (b) is tPAgp120 or tPAgp160.

73. A polynucleotide which is non-replicating in eukaryotic cells *in vivo*, comprising sequences encoding:

- a) a eukaryotic transcription initiation signal;
- b) a first HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the HIV gene ORF does not depend on availability of the HIV REV gene product;
- c) a sequence which operates as an internal ribosome entry site (IRES) 3' to the translation stop codon of the first HIV ORF;
- d) a second HIV gene open reading frame (ORF) preceded by a heterologous leader sequence such that expression of the second HIV gene ORF does not depend on availability of the HIV REV gene product; and
- e) a transcription termination signal 3' to the translation stop codon of the second HIV gene ORF.

74. A polynucleotide which, upon *in vivo* introduction into a mammalian cell, is non-replicating and induces the co-expression in the cell of at least two gene products, the polynucleotide comprising a first transcriptional promoter which operates in eukaryotic cells upstream from, and in transcriptional control of, a first cistron, a second cistron downstream from the first cistron, under transcriptional control either of the first transcriptional promoter or under control of a second transcriptional promoter, optionally, a third cistron downstream from the second cistron, under transcriptional control either of the first transcriptional promoter or under control of the second transcriptional promoter, or under control of a third transcriptional promoter, and a transcriptional terminator following each of the first, second and third cistron, unless said first cistron or second cistron is followed by a second cistron or third cistron, respectively, which lacks its own transcriptional promoter; wherein each of the first, second and optionally third cistrons encode a combination of any two to three of the following:

- 1) tPA-gp120_{MN};
- 2) gp160_{IIIB}/IRES/REV_{IIIB};
- 3) gp160_{IIIB};
- 4) REV_{IIIB}
- 5) tat/REV/gp160;
- 6) REV/gp160;
- 7) gp160_{MN};
- 8) gp160 from a clinical HIV isolate;
- 9) nef, using the gene from a clinical HIV isolate;
- 10) gag_{IIIB};
- 11) tPA-gp120_{IIIB};
- 12) gp160 with structural mutations selected from the group consisting of V3 loop substitutions from a clinical HIV isolate; several mutations on several constructs such as variable loop removal, Asn mutations to remove steric carbohydrate obstacles to structural, neutralizing antibody epitopes; and CD4 binding site knockout mutants;
- 13) gp41 with a signal peptide leader sequence;
- 14) gag/REV/gp160;
- 15) rev: for gp160 and gag dicistronics;
- 16) a nucleotide sequence encoding B7;
- 17) a nucleotide sequence encoding GM-CSF;
- 18) a nucleotide sequence encoding interleukin sequences; and,
- 19) a nucleotide sequence encoding tumor associated antigens;

75. A polynucleotide construct selected from the group consisting of V1Jns-(tat/rev SD), V1Jns-gp160_{IIIB}/IRES/rev_{IIIB} (SD), V1Jns-gag-prt_{IIIB} (SD), V1Jns-gag-prt_{IIIB}, V1Jns-tPA, V1Jns-tPA-gp120_{MN}, V1J-SIV_{MAC251}p28 gag, V1J-SIV_{MAC251}nef, and V1Jns-tat/rev/env.

76. The polynucleotide of Claim 50 wherein the first cistron contains an HIV *gag* gene or portion thereof which encodes a *gag* immunogenic epitope, the second cistron encodes a cytokine, and the third cistron encodes a T-cell costimulatory element, wherein the first, second and third cistron may be presented in any combination.

77. The polynucleotide of Claim 76 wherein the second cistron encodes an interleukin, an interferon, or GM-CSF, and the third cistron encodes a B7 protein.